β-Lactam Cell Wall Inhibitors

Introduction

Penicillin was the first antibiotic. Penicillin is a **β-lactam**. β-lactams are called that because they have a β-lactam **ring** in their structure. We use the term "*β-lactam*" for the broad class of all antibiotics that contain a β-lactam ring. This includes penicillins, cephalosporins, monobactams, and carbapenems. All groups contain the β-lactam ring, a sulfur, and some side groups. Each class of medication differs in the side groups, how the sulfur is incorporated into the structure, and how many R groups there are. The β-lactam ring is what binds to penicillin-binding proteins (the intended effect of β-lactams) and is what β-lactamase cleaves (a mechanism that bacteria have developed to resist being killed by penicillins). For ease of discussion, "penicillin-binding protein" and "transpeptidase" (the final step in cross-linking of peptidoglycan chains) are the same thing.

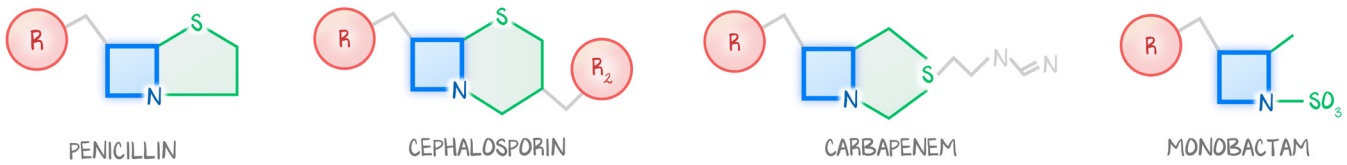


Figure 2.1: β-Lactam Antibiotics
All β-lactam antibiotics share a very similar structure, consisting of an R group (red), a β-lactam ring containing a nitrogen (blue), and some form of a sulfur moiety (green). The β-lactam ring is the active site that targets penicillin-binding proteins, and is also the site of catabolism by β-lactamases. The sulfur moieties are responsible for allergic reactions. Monobactams have the least-similar sulfur structure, and therefore also the lowest cross-reactivity in regard to allergic reactions.

The goal of β-lactams is to disrupt the synthesis of the peptidoglycan cell wall. For Gram-positive organisms, the drug has direct access to the cell wall. For Gram-negative organisms, there is the outer plasma membrane that the drug must first diffuse through to access the peptidoglycan cell wall in the periplasmic space. We will explore how the size of the molecule (the R groups) modify how the β-lactam functions. We will also explore how resistance develops to each drug based on whether or not there is an outer plasma membrane.

We first discuss β-lactam resistance, then penicillins in general, walk through the specific penicillin classes, then change gears and broaden the coverage with cephalosporins and monobactams, and close with carbapenems. Other inhibitors of the cell wall and plasma membrane are discussed in the next lesson, *Cell Wall Inhibitors (Not β-Lactams)*.

Resistance Mechanisms to β-Lactams

Because Gram positives lack an outer membrane, they are forced to deal with penicillins via the production of β-lactamase and alteration of the penicillin-binding proteins only. Because Gram negatives have an outer membrane, they are able to utilize β-lactamase and alter PBPs, but also get to use membrane porin solubility and effusion mechanisms.

β-Lactamase. β-lactamase is an enzyme secreted by the bacterium into the cell wall space, which results in **hydrolysis** of the **β-lactam ring**, rendering the β-lactam useless. The genes for β-lactamase are carried on **plasmids**. When an organism possesses the ability to cleave the β-lactam ring, **ALL β-lactams are rendered useless**. While not all β-lactams have the same susceptibility to β-lactamase, you should see β-lactamase as the resistance method that can knock out not just penicillins as a class, but all β-lactams.

Alteration of PBPs. To keep the discussion simple, we have said that PBP is transpeptidase, but really any number of enzymes involved in cell wall synthesis (outside the plasma membrane) can be

affected. Penicillins have an affinity for those enzymes (PBPs) because of the structure of the β-lactam and the structure of the PBP. If bacteria develop mutations in the PBP such that the PBP's active site for transpeptidation is the same, but the binding site for penicillin is changed, the result will be less effective penicillin. Penicillins work because they bind PBPs more readily than PBPs bind their substrate. If that changes, PBPs can still complete cross-linking in the presence of β-lactams, and the β-lactams become useless.

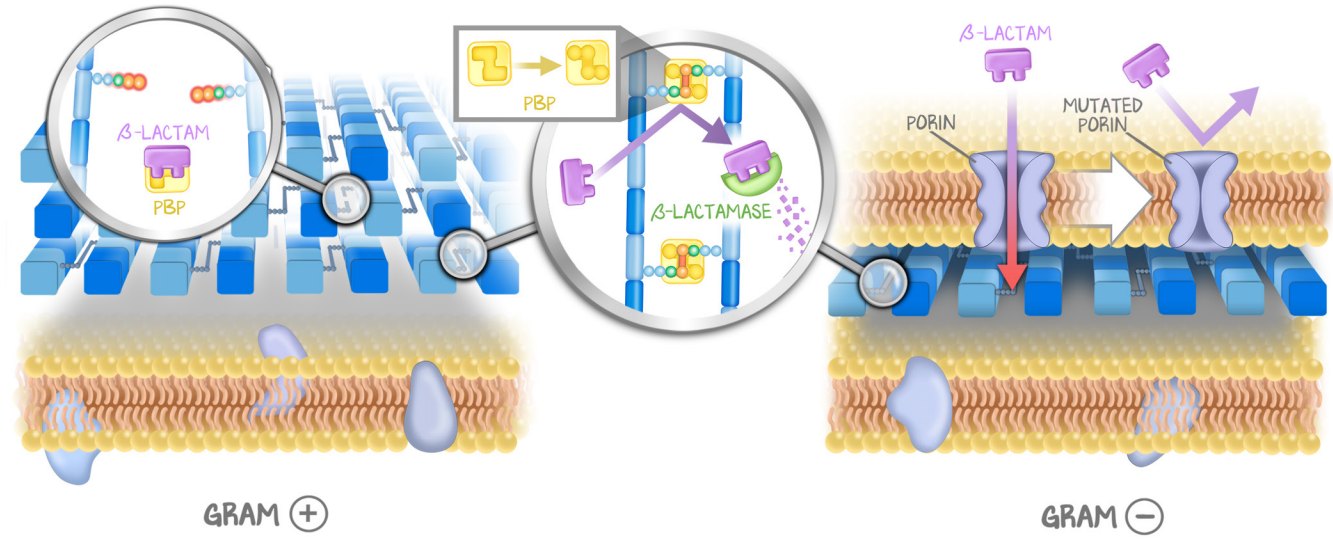


Figure 2.2: Gram Positive vs. Gram Negative
Gram positives have no outer membrane, so penicillins have direct access to the cell wall. They can only develop β-lactamase and change their PBPs. Gram negatives have a cell wall, so can develop β-lactamase and change their PBPs. Gram negatives also have an outer plasma membrane, preventing certain penicillins from reaching the cell wall, and allowing for mutations in porins or effusion pumps as additional resistance mechanisms.

Decreased permeability to the drug refers to the **Gram-negative organisms** that have an outer plasma membrane through which penicillins must pass to impact the smaller peptidoglycan layer in the periplasmic space. Mutations in porins may prevent entry of a drug to the periplasmic space where the penicillin-binding proteins are. In a similar vein, **effusion** is the active pumping of the active drug across the outer membrane and back into the extracellular space. Both reduced access and increased egress are resistance patterns available only to Gram-negative bacteria.

Penicillins in General

Mechanism. All penicillins work the same way. They are **D-Ala-D-Ala analogs** that bind to the **transpeptidases** involved in cell wall synthesis. Transpeptidases catalyze the cross-linking of the peptidoglycan chains. If these proteins are bound to a β-lactam antibiotic instead, they can't be used to connect the peptidoglycans, and the cell wall falls apart. When first discovered, they were in relationship to the mechanism of action of penicillins, so they were given the name **penicillin-binding proteins** (details and assumptions discussed above). Without cross-linking layers of the cell wall together, there is no stability of the wall, which results in **osmotic flow of water** and **lysis** of the bacteria. Because the mechanism of cross-linking failure relies on inhibiting the synthesis of new cell walls, penicillins do require a fairly active bacteria (rapidly growing organisms making lots of new cell wall are the most affected). And because it targets the formation of a peptidoglycan layer, Gram-positive organisms are going to suffer the greatest. The outer membrane of Gram-negative organisms both provides a barrier to accessing the peptidoglycan cell wall and serves as extra structural osmotic support if that peptidoglycan cell wall is compromised.

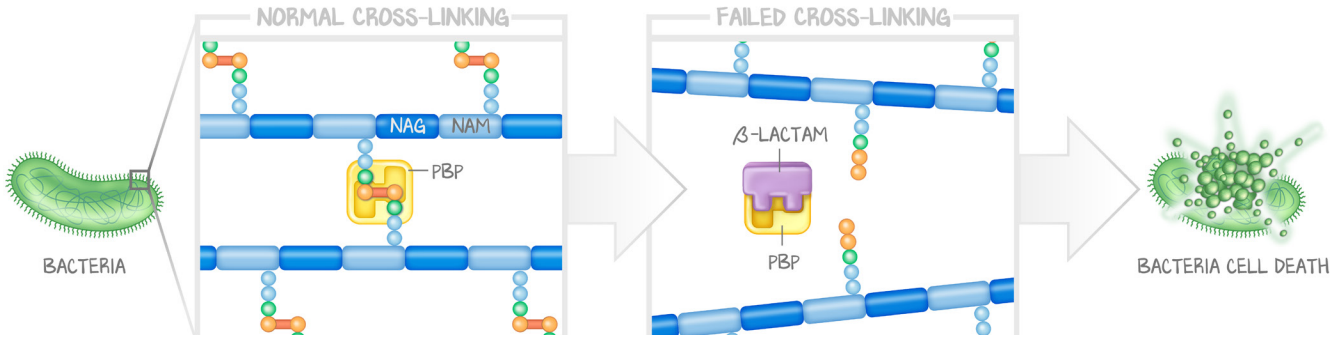


Figure 2.3: Mechanism of Penicillins
Penicillin binds to penicillin-binding protein, the transpeptidase that completes cross-bridge linking of peptidoglycan chains. This results in the loss of structural integrity and resistance to osmotic changes, which result in cell death.

Side Effects = Rash and Anaphylaxis. Allergic reactions are the main worry for penicillin administration. These antibiotics are made by fungus (**protein** of another organism), are **large,** and contain **sulfur**. Sulfur, large, and protein scream possible allergic reaction. All penicillins can cause a spectrum of allergic reactions, from **rash** (mild reaction) to a reaction so severe as to cause **anaphylaxis**. If ever a patient has an **anaphylactic reaction** to any one penicillin, he will also have **cross-reactivity** to any other penicillin, so penicillins must be avoided. There may also be cross-reactivity with other β-lactams. The severity of the allergic reaction to penicillin will determine the safety of another β-lactam (discussed later under cephalosporins). Finally, penicillins are **water soluble**, and so are excreted by the **kidney**, and so can cause **renal failure** and must be renal-dose adjusted. The more synthetic, broader-spectrum penicillins have a higher risk of **interstitial nephritis** (piperacillin/tazobactam).

Natural Penicillins: The Original Pen G and Pen V

Penicillin G (IV and IM) and penicillin V (oral) are the prototypical β-lactam penicillin antibiotics. They are produced by a fungus. All synthetic penicillins are based on them, varying only in their R group. Penicillin, on its own, is the empiric treatment for **primary syphilis**. It is never the empiric choice for any other infection. If ever a culture shows penicillin sensitivity, it is the drug of choice. Figure 2.4 shows the similarities and differences between synthetic penicillins and the consequences of those modifications.

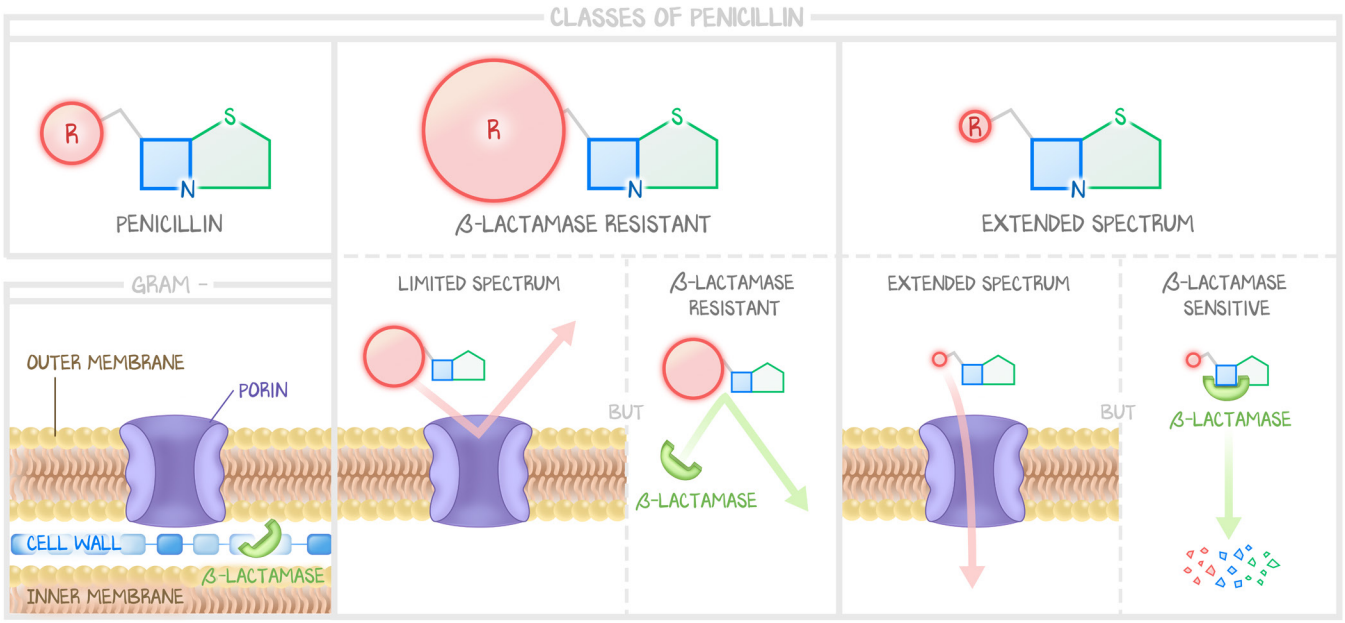


Figure 2.4: Classes of Penicillins
Larger, bulkier R groups make the penicillin resistant to β-lactamase, having the R group physically in the way of the active site for β-lactamase. But being bulkier prevents penetration through Gram-negative rods' outer membranes, narrowing the spectrum. Smaller, more efficient R groups make the penicillin easily slip through a Gram-negative rod's porins, but also make it more susceptible to β-lactamase.

As we explore the different classes of penicillins, pay attention to their **spectrum** and **sensitivity to β-lactamase** (and therefore the need for β-lactamase inhibitors to be added).

β-Lactamase-Resistant Penicillins ("Antistaphylococcal Penicillins")

Staph. aureus has a particularly strong β-lactamase and is immune to most penicillins because of it. By synthesizing a penicillin with a bulkier R group, we provided a physical barrier to *Staph. aureus* β-lactamase. The bulky R group (it is REALLY bulky) also renders the penicillin we synthesized this way too large to fit through a port, rendering this class completely useless against Gram-negative organisms. *Strep.* species do not have an outer membrane, which means these penicillins could work against *Strep.* species. Do not use these β-lactams for strep. Doing so will breed β-lactamase-resistant strep, just as we did for staph.

The first synthetic penicillins traded **spectrum** in order to obtain **resistance to β-lactamase**. These are called "antistaphylococcal penicillins" because they work only on *Staph. aureus*. Their β-lactamase resistance is derived from a bulky R group that limits β-lactamase access to the β-lactam ring. They are used to treat **methicillin sensitive *Staph. aureus*** (MSSA). They are never the empiric choice in clinical practice, given the high prevalence of methicillin-resistant *Staph. aureus* (MRSA). However, they are far better tolerated than anti-MRSA antibiotics, and should a culture reveal MSSA, these should be employed. **Nafcillin** is the **IV form**, while oral formulations are **oxacillin** and **dicloxacillin**.

Nafcillin's metabolites (also oxacillin) are **not water soluble** and are only **lipid soluble**, so nafcillin's elimination is hepatic, its metabolites excreted in **bile**. It is administered every four hours, and is used for bloodstream infections and endocarditis. It is not cleared by the kidneys, so renal dosing is not required.

Methicillin is never used clinically. It is used only in the laboratory to assess *Staph. aureus* susceptibility to these β-lactamase-resistant penicillins. Using β-lactamase-resistant penicillins bred MRSA. The mechanism by which *Staph. aureus* became resistant is discussed in the next lesson, Antibacterials 3: *Cell Wall Inhibitors (Not β-Lactams).*

β-LACTAMASE-RESISTANT (STAPH PCNS)		EXTENDED-SPECTRUM AMINOPENICILLINS (GN PCNS)	
Nafcillin	IV, hepatic elimination	Ampicillin	IV, amp/sulbactam
Oxacillin	PO, hepatic elimination	Amoxicillin	PO, amox/clav

Table 2.1: Examples of Different Penicillin Classes

Aminopenicillins, aka Extended-Spectrum β-Lactamase-Sensitive Penicillins

The **am**inopenicillins are named **ampicillin** and **amoxicillin**. The exact opposite to the antistaphylococcal penicillins, the amino penicillins traded **β-lactamase resistance** in order to **expand their antibiotic spectrum**. They gained the ability to **treat Gram-negative rods**. Like anti-staph penicillins, these also treat *Strep.* species, and should be chosen in most instances if strep is suspected. The aminopenicillins are used as empiric coverage for non-staph skin infections; for infections of the eyes, ears, nose, and throat; and as coverage for infections commonly caused by Gram-negative rods, such as urinary tract infections.

Their enhanced ability to target Gram-negative rods comes from the small size of their R group, which enhances **penetration through porins** and improves their ability to diffuse into the periplasmic space. But there is a trade-off. By becoming smaller they became easier for β-lactamase to bind to and inactivate.

The genes for β-lactamase are carried on plasmids. This form of antibiotic resistance is easy to develop, and plasmids make it easy to share. And so amoxicillin and ampicillin are often used in combination with a **β-lactamase inhibitor**. This was one of the examples of synergy in the first lesson. There are two you should know: clavulanate and sulbactam. Learning the generic shorthand, "*amox/clav*" (PO amoxicillin/clavulanate) and "*amp/sulbactam*" (IV ampicillin/sulbactam), helps not only to avoid succumbing to trade names, but also helps keep track of which β-lactamase inhibitor goes with which penicillin, and by which route they are most commonly administered.

Because of their vulnerability to β-lactamase, aminopenicillins are generally ineffective against *Staph. aureus*. And thus, the division between the extended-spectrum penicillins which fight Gram negatives and strep but don't fight *Staph. aureus*, and the β-lactamase resistant-penicillins which fight only *Staph. aureus* but can't fight Gram negatives and should not be used for strep. Even when the β-lactamase inhibitor is added to the penicillin, *Staph. aureus* is just too much of a badass to succumb.

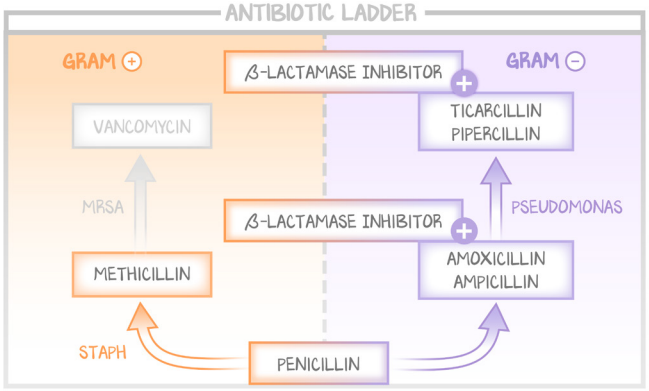
We're building the antibiotic ladder without your knowing it. The ladder has amoxicillin and ampicillin as Gram-negative and strep coverage, and does not account for their anaerobic coverage. While generally not the go-to antibacterials to empirically cover anaerobes, amoxicillin and ampicillin do have decent anerobic coverage as well as their Gram-negative coverage.

Antipseudomonal Penicillins

The aminopenicillins do not treat *Pseudomonas*. The antipseudomonal penicillins are **super-extended spectrum** and, in addition to the Gram negatives and *Strep*. species treated by aminopenicillins, these penicillins can also treat Pseudomonas and **anaerobes**. Antipseudomonal penicillins should be chosen only to empirically treat a pseudomonal infection, not because they are convenient and cover "a bunch of bugs." Their convenience—they treat everything except *Staph. Aureus*—makes them widely overused to treat most any infection, even if not pseudomonal. Their overuse—overtreating every infection except pseudomonal—breeds antibiotic resistance. The antibiotic resistance it breeds is against β-lactamases in general.

Their empiric use is reserved for infections with high risk of *Pseudomonas* and should be chosen only when *Pseudomonas* is a possibility. This means **healthcare-associated pneumonias** and **diabetic foot wounds**.

The only antipseudomonal penicillins available on the market are those that are combined with a β-lactamase inhibitor. They are available intravenously only. The two drugs to know are "pip/tazo" (**piperacillin/tazobactam**) and "tic/clav" (ticarcillin/clavulanate).



BUG	DRUG	DRUG
Pseudomonas	Pip/tazo	Tic/clav
Gram –	Amp/sulb	Amox/clav
Staph	Nafcillin	Oxacillin
Syphilis	PCN	

Figure 2.5: The Antibiotic Ladder
This is the beginning of the antibiotic ladder. It employs only what you've learned so far—penicillins—and should serve as a review for the lesson so far, transitioning away from penicillins, and into the rest of the β-lactams.

Cephalosporins

Cephalosporins differ from penicillins in the **size of their sulfur ring group** (making them inherently more resistant to β-lactamase) and the **addition of a second R group** (see Figure 2.1). It is that second R group which allows there to be such variation in the bacteria that cephalosporins treat. Cephalosporins' mechanism of action, resistance pattern, clearance, and allergic reactions are essentially identical to those of penicillins. Given their similarity, there is concern for cross-reactivity. Empirically, there has been only about a 10% risk of cross-reactivity from a penicillin allergic reaction to cephalosporins. This means two things. First, **if ever there were an anaphylactic reaction to any penicillin, you must not use any cephalosporins**, as 10% risk is too high for a life-threatening reaction. Second, any nonanaphylactic reaction reported by a penicillin should NOT preclude the use of a cephalosporin, the benefit of the cephalosporin outweighing a 10% chance of a non-life-threatening reaction.

Cephalosporins are categorized by their generation, which corresponds to the bugs they treat. You do NOT need to know what the biochemical R group variation is. You DO need to able to identify the cephalosporin name, correspond that to the generation, and then know what bugs it is most likely to treat.

First-generation cephalosporins (cefazolin, cephalexin) treat Gram-positive cocci. They are effectively penicillin G substitutes that are β-lactamase resistant (so can cover MSSA and strep). The main use is **prophylactic intravenous cefazolin** before and after surgery to reduce the risk of wound infection. The bugs on the skin are strep and staph. **Cephalexin** is the oral form that can be used to treat outpatient skin infections.

Second-generation cephalosporins have fallen out of favor in general. Their use declined in general because first-generation cephalosporins are better at killing Gram positives, third-generation cephalosporins are better at killing Gram negatives, and *Bacteroides* species has developed resistance to cephalosporins. *Bacteroides*? *Bacteroides* is a Gram-negative anaerobe. The cephamycins, a subclass of second-generation cephalosporins, cefoxitin and cefotetan, are the only cephalosporins that confer any anaerobic coverage. Their only utility was the anaerobic coverage. The bugs they were used to treat have developed resistance. Therefore, the second-generation cephalosporins are mainly a historical placeholder.

Third-generation cephalosporins are the **intravenous empiric antibiotics** of choice for many infections. These are the cephalosporin analogs to aminopenicillin + β-lactamase inhibitor. In this sense, they treat Gram **negatives** (except *Pseudomonas*) and Strep. **species,** but are ineffective against *Staph. aureus*. The third-generation cephalosporins also **cross the blood-brain barrier**. Ceftriaxone is one of the most commonly used antibiotics in hospitalized patients, being used empirically for meningitis, pneumonia, skin infections not staph, and urinary tract infections. This, unlike pip/tazo's widespread use, is appropriate empiric coverage. But because **ceftriaxone** is the cephalosporin of choice in practice, you must be able to identify other third-generation cephalosporins for your test. Cefotaxime is the one to know. Ceftazadime, "ceftaz," is also a third-generation cephalosporin, but has the special property of being antipseudomonal. Learn ceftriaxone for life, cefotaxime for the exam, and ceftazidime as the exception that treats *Pseudomonas*.

Fourth-generation cephalosporins are antipseudomonal and are analogs to the antipseudomonal penicillin + β-lactamase inhibitors. Like those penicillins, cefepime, THE fourth-generation cephalosporin, also has anaerobic coverage. See pip/tazo and **cefepime** as the same drug which can be used under the same conditions, interchangeably with each other. In practice, however, cefepime is usually reserved for empiric coverage of patients with **neutropenic fevers** only.

There is a fifth-generation cephalosporin that fights MRSA, ceftaroline. It covers staph but not *Pseudomonas*. Never use this drug.

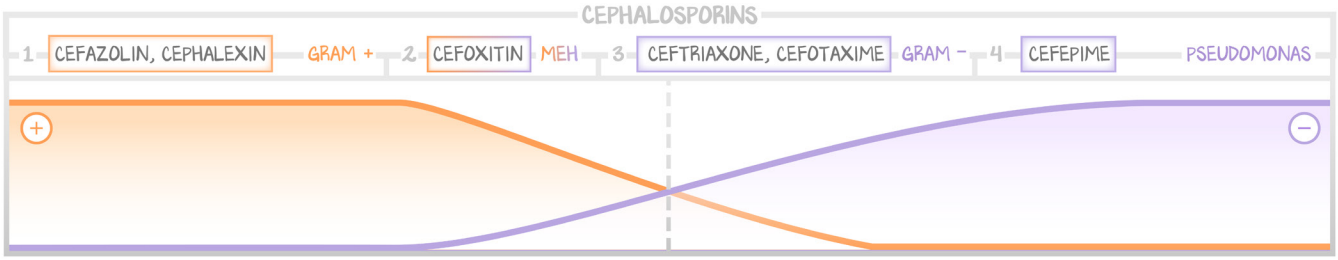


Figure 2.6: The Cephalosporin Foundation
This is a summary image that goes beneath the antibiotic ladder, acting as its foundation. Early-generation cephalosporins are used for Gram-positive coverage, later generations Gram-negative.

Carbapenems

Carbapenems have their sulfur group externalized (see Figure 2.1). They have massively extended spectrums. They **do not treat staph**, but can treat *Pseudomonas*, strep, and aerobes. They should be considered "higher-on-the-ladder" when compared to the antipseudomonal penicillins and antipseudomonal cephalosporins. They are reserved for **empiric pseudomonal coverage when penicillin anaphylaxis has occurred** (thereby rendering penicillins and cephalosporins unusable), and for treatment of **extended-spectrum β-lactamase-(ESBL)**-producing organisms such as ESBL *E. coli*. That is, the use of antipseudomonal antibiotics has bred an organism that has such a strong β-lactamase that no other β-lactam can work.

Ertapenem is the black sheep. It doesn't do anything it is supposed to. It does **not** cover *Pseudomonas* or *Enterococcus*. There is never a reason to choose ertapenem.

Imipenem/cilastatin and **meropenem** are the drugs of choice. They are intravenous only. Imipenem requires cilastatin, an inhibitor of a dehydropeptidase in the brush border of the kidney, permitting imipenem/cilastatin to avoid both nephrotoxicity and rapid clearance. They can cause nephrotoxicity, but are generally well tolerated. Their use should be avoided except when absolutely necessary—using them risks antibiotic resistance.

Monobactams

The final class of β-lactams is the monobactam, which has no sulfur ring at all (see Figure 2.1). **Aztreonam** is the **most resistant β-lactam** there is. Aztreonam loses the large bulkiness of the β-lactam structure, and so has **no cross-reactivity** with penicillins. What that does, however, is cause **loss of spectrum**. Aztreonam is used exclusively for Gram-negative rods, especially ***Pseudomonas*.** It is an alternative to carbapenems when allergic reactions are the issue.

ANTIBIOTIC	EXAMPLES	USED TO TREAT
Penicillin	Pen G, Pen V	Syphilis
β-lactamase resistant	Nafcillin, oxacillin	*Staph. aureus*
Extended spectrum	Amoxicillin, ampicillin	Gram negatives and strep
Extended spectrum + β-lactamase inhibitor	Amoxicillin/clavulanate Ampicillin/sulbactam	Gram negatives and strep
Antipseudomonal	Pip/tazo	*Pseudomonas*
1st-generation cephalosporin	Cefazolin, cephalexin	Gram positives
2nd-generation cephalosporin	Cefoxitin	None
3rd-generation cephalosporin	Ceftriaxone, cefotaxime	Gram negatives and strep
4th-generation cephalosporin	Cefepime	*Pseudomonas*
Carbapenems	Imipenem/cilastatin Meropenem	VRE, ESBL, *Pseudomonas*
Monobactams	Aztreonam	Gram-negative rods only

Table 2.2: Summary Table